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Alkylphenol and Alkylphenol-Ethoxylates in Carp, Water, and Sediment from the Cuyahoga River, Ohio

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The occurrence of alkylphenol and alkylphenol ethoxylates (APEs) was determined over a 74-mile length of the Cuyahoga River, Ohio. Measurable levels of both the octyl and nonyl forms of these abundantly used nonionic surfactants were observed with the nonylphenol (NP) plus nonylphenol ethoxylates (NPEs) typically accounting for greater than 90% of the total APEs in each sample. For all media (water, fish, and sediment) the total NPE (NP + NPE) concentrations were higher in the more urbanized downstream section of the river. Maximum water and fish values were observed immediately downstream (2.1 miles) from the discharge of the Akron WWTP located 35.31 miles from the river mouth and the sediment maxima occurred at the most downstream site near Cleveland. The ranges in concentration for total NPEs and their ethoxylate (EO) makeup were as follows: 32−920 ug/kg wet wt (NP 0 to 2 EO) for carp; 0.13−1.0 ug/L (NP 0 to 3 EO) for water; and 250−1020 ug/kg dry wt (NP 0 to 5 EO) for sediment. When the higher ethoxymers (NP 6 to 17 EO) were added to these sediment totals, the average total estimated NPE concentrations were 1.3−1.8 times higher.

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Introduction

Alkylphenols and alkylphenol-ethoxylates (APEs) are established markers of industrial and municipal pollution (*1*−*3*). The compounds find their way into the environment in their original forms and as natural degradation products of the parent alkylphenol ethoxylates that are popularly used in commerce for their surfactant properties. Complete ethoxy removal from this polymer class of chemicals yields either nonylphenol (NP) or octylphenol (OP). These are the base structures of the two most heavily manufactured and used members of this nonionic class of surfactants. Both of these compounds are established endocrine disrupter chemicals (*4*). As the ethoxy substitution increases from 1 to 20 units (the range of ethoxy substitutions most commonly found in commerce (*5*)) the toxicity and endocrine activity of both of them decreases. Owing mostly to the greater use of the nonylphenol form, this is the homologue group most reported as an environmental contaminant. For example, several studies have confirmed the presence of 4-nonylphenol, 4-nonylphenol monoethoxylate (NP1EO), 4-nonylphenol diethoxylate (NP2EO), and 4-nonylphenol triethoxylate (NP3EO) in river waters downstream of wastewater treatment plants (WWTP) (*6*−*8*) and industrial outfalls that utilize NP-ethoxylates in their industrial processing, such as wool rendering operations in the United Kingdom (*9*). The greatest quantity of data for water concentration of APEs exists for the NP, which is the easiest to detect by gas chromatography/mass spectrometry (GC/MS). However, when environmental samples are analyzed for the lower ethoxymers, especially NP1EO and NP2EO, they are usually found and sometimes they are present at even higher levels than NP (*7, 8*). Numerous data also exist for the occurrence of nonylphenols in sediment of rivers and lakes (*10*−*14*) and in sediment of coastal waters (*6, 2, 15*).

Nonylphenol and nonylphenol ethoxylates are frequent contaminants in fish, especially in waters near known areas of wastewater and industrial discharge (*16*−*18*). One of the earliest reports for accumulations of NPEs by fish was provided by Ahel et al. in 1993 (*16*) in Switzerland. They confirmed the presence of the NPEs in water from a stream and in fish collected from the same stream. Converting their values to wet weight, the estimated whole-fish concentrations for *Squalis cephalus* was 0.035 μg/g wet wt NP; 0.220 μg/g NP1EO; 0.160 μg/g NP2EO or a total of these t-NPE of 0.410 μg/g wet wt. Data on residues of alkylphenols in fish collected from North American waters have only been infrequently reported in the literature. To the present, only five reports have been published (*12, 19*−*22*), involving NP (0−5) EO and OP. Of these studies, only the Kannan et al. study (*22*) involved fish, water, and sediment samples together in order to evaluate APEs behavior in different environmental compartments, but they found little or no contamination. Such information however is clearly needed for policymakers to begin to evaluate the relative risks of this group of chemicals.

This study was conducted to determine the abundance of alkylphenols (nonyl- and octylphenol) and nonyl- and octylphenol ethoxylates, NP&OP1 to 5EO in carp, water, and sediment over an extensive length of the Cuyahoga River. The Cuyahoga River can be divided into three regions, the upper section, a middle portion, and the lower region. Historically, a natural feature of the upper section was a rich

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TABLE 1. Description of Cuyahoga River Sampling Sites

Cuyahoga River sites	site name	mile point	latitude	longitude
site 1	Eldon Russell Park	83.93	41°25′41″	81°09′15″
site 2	Standing Rock	56.1	41°09′58″	81°21′01″
site 3	Upstream-Little Cuyahoga River	42.73	41°07′05″	81°31′21″
site 4	Downstream-Little Cuyahoga River	41.95	41°07′23″	81°32′00″
site 5/6	Downstream − Akron WWTP	35.31	41°10′53″	81°35′00″
site 7	Upstream Southerly WWTP	11.33	41°25′03″	81°38′30″
site 8	Downstream Southerly WWTP	10.35	41°25′14″	81°39′30″

abundance of spongy wetlands that provided rich habitats and many bogs and fens. While the area still retains some of these qualities, many of them have been drained and many tributaries are now channelized. The upper portion transitions into the cities of Kent, Cuyahoga Falls, and Akron where urban and industrial development begin to impact the river. Several dams have been constructed over this area of the River. The uppermost dam, just upstream of Kent, identifies the beginning of the middle portion of the Cuyahoga watershed. These dams, along with the combined sewer overflow (CSO) discharges especially in the Akron Area and CSO contributions from the Little Cuyahoga River, have seriously degraded aquatic life habitat of the Akron portion of the Cuyahoga River (*23*). The middle portion lies predominantly in Summit County, where it encompasses much of the Cuyahoga Valley National Recreation Area, the largest park in the watershed. This area occupies about 22 miles of the River from Akron to Cleveland, extending from MP (Mile Point) 39 to 13. The lower portion of the Cuyahoga River watershed is the region from Big Creek (MP 7.2) to Lake Erie. This area of the river is entirely within the city of Cleveland and has the most intensive industrial development for the entire river. The river is typically less than 30 m in width, and the bottom composition varies from rocky to generally fine-grained sediment in areas of deposition. An important goal of this study was to attempt to describe the pattern of distribution of APEs over the river in order to better identify possible loading sources. One of the more puzzling recent pollution-related problems with the Cuyahoga River is the fact that fish species near the top of the food chain are not establishing themselves in the middle section of the river particularly from the confluence with the Little Cuyahoga to the river mouth. Much data collected by Ohio EPA suggest that a significant source of impairment to fish communities in this region could be coming from the Little Cuyahoga River (*23*). The Little Cuyahoga River watershed contains the majority of current and past industrial facilities in the Akron area and receives discharges of untreated domestic and industrial wastewater from approximately 33 combined sewer overflows. There are at least 19 potential abandoned hazardous waste sites in Akron, and 10 of these are close to the banks of the Little Cuyahoga River that passes directly through the city of Akron. In addition to these pollutant sources, the Cuyahoga River also receives effluent from the 90 mgd (million gallon per day) Akron Wastewater Treatment Plant (WWTP), which during low flow conditions can contribute approximately 70% of the flow in the river. Approximately 10% of this WWTP flow is derived from industrial sources (*24*). This treatment plant is the highest WWTP dischargers over the middle portion of the river, the next highest is the Kent WWTP at 5 mgd.

The study was conceived and designed by the staff of Ohio EPA and was part of a larger program of sampling and analysis on this river that included organic and inorganic water and sediment pollutant scans. Information about these other data can be obtained from the staff of the Ohio Environmental Protection Agency, Groveport, OH 43215 (*24*). In addition to the chemical analyses, the levels of three specific hormones (vitellogenin, 17-β estradiol, and 11-keto testosterone) were also determined on each individual fish sample reported below. These data will be reported in subsequent publications.

Experimental Section

Sample Collections. Common carp, *Cyprinus carpio*, were collected July 11−13, 2000 by electroshocking. The fish were held briefly for biological processing (*25*), after which each carp sample was wrapped in aluminum foil, sealed in a plastic bag, placed in dry ice, and brought back to the Ohio EPA Field Facility for placement in a freezer. Equal numbers of male and female fish (total of 12) were sought at each of the following Cuyahoga River locations: site #1, Eldon Russell Park, MP 83.93; site #2, Standing Rock, MP 56.1; site #3, Upstream-Little Cuyahoga River, MP 42.73; site #4, Downstream-Little Cuyahoga River MP 41.95; site #5/6 (field duplicate site), Downstream of the Akron WWTP, MP 35.31; site #7, Upstream-Southerly WWTP, MP 11.33; and site #8, Downstream-Southerly WWTP, MP 10.35 (Table 1 and Figure 1).

Water and sediment samples were obtained at the above locations and two samples of each at site #5/6 for duplicate analyses. Surface water samples were collected starting at the upstream site, Eldon Russell Park, at 12:45 p.m. on July 7, 2000, and proceeded downstream, with the final samples collected downstream of the Cleveland Southerly WWTP outfall on July 8th, 2 p.m. EST. The water sampling procedure involved wading into the river and dipping each bottle below the surface and allowing it to fill. Four 1-L bottles were obtained at each site; 8 bottles were obtained at the duplicate field site, site 5/6. These samples were collected during low-flow conditions (28% and 43%, respectively, of the average yearly upstream (5600 L/s) and downstream (24 000 L/s) flows for that year) to assess the impacts of worst-case e.g., low flow, conditions in the river. After collection these samples were shipped on ice to USDA in Beltsville, Maryland.

River sediment grab samples were collected from July 13 to July 14, 2000, from fine-grained depositional areas of the Cuyahoga River by using chest waders. Disposable Teflon scoops were used to transfer sediment directly from the river bottom (7−25 cm thick) into a pan, wherein they were homogenized and placed into glass jars with Teflon-lined lids. The samples were kept on ice and brought back to the Ohio EPA Field Facility for placement in a freezer. For shipment to Beltsville, MD, the fish and sediment samples were shipped in coolers with dry ice under full chain of custody. On arrival they were again placed in a freezer for maintenance at −20° C until analysis.

Standards and Reagents. Analytically pure standards were obtained commercially for NP2EO from Aldrich Chemical Co., Milwaukee, WI (95% purity), octylphenol from Aldrich Chemical Co. (97% purity, CAS 140-66-9), and nonylphenol from Schenectady International, Schenectady, NY (purity greater than 95%, CAS 84852-15-3). n-NP, n-NP3EO, $^{13}C_6$-NP and $^{13}C_6$-NP-ethoxylates were received as gifts from P. L. Ferguson, State University of New York at Stony Brook (*15*, *26*). Individual NP-ethoxylates (NP1, NP3, to NP5-ethoxymers) and OP-ethoxylates (OP1 to OP5-ethoxymers) were purified

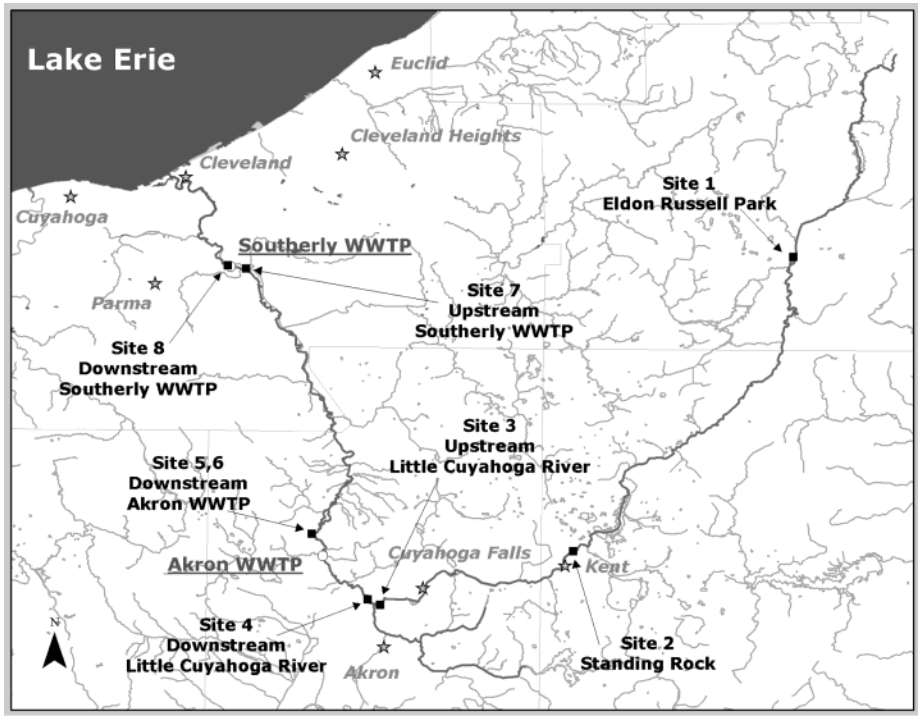


FIGURE 1. Cuyahoga River with sampling sites designated.

from selected commercial ethoxylate mixtures using silica gel chromatography (*19*, *27*). PCB 204, 4-propylphenol (CAS no. 645-56-7), and 4-*n*-heptylphenol (CAS no. 1987-50-4) were all used as internal standards. Solvents were all pesticide grade (Burdick & Jackson, Honeywell Intl. Inc., Muskegon, MI). Deionized, carbon-free water (DI water) was purified by a NANOpure water purification system (Barnstead International, Dubuque, IA). Anhydrous sodium sulfate (J. T. Baker, Paris, KY), granular powder, was oven baked for 4 h at 400 °C. Ammonium acetate was obtained from Aldrich (purity 99.99%) and stored in a desiccator. Pentafluoro benzoyl chloride (PFBC) for chemical derivatization was obtained from Aldrich Chemical Co. The pyridine solution, used in derivatization reactions, was analytical grade from Baxter Healthcare Corp., Muskegon, MI, and a basic, pH 8.5, solution of a borate (Fisher Scientific, Pittsburgh, PA, (4 g Na-borate/100 mL), was also used in the derivatization procedure. Special attention was paid not to use any detergent or plastics in any stage of the following procedures since they are possible sources of nonylphenol.

Water Preparation Procedures. The water was received on ice in 1-L glass bottles (4 per sample) and stored for 2 days at 4 °C prior to analysis. The following preparation procedures are described more fully in ref *27*. For analysis of the water from each sampling site, each set of four 1-L jars was filtered into a 4-L amber glass bottle (previously baked for 4 h at 450 °C). This filtration was performed with 1-μm Multigrade GMF 150 filters connected in series with 0.7 μm GF/F filters (both from Whatman Inc., Clifton, NJ). One extra 4-L bottle was filled with carbon-free water and spiked with 50 μL of a standard solution of NPEs and OPEs. To generate a field blank, an empty 1-L bottle was transported to and from the field. This bottle was rinsed in the laboratory with 4 L of carbon-free water, which then became the field blank. Each sample was extracted using solid-phase extraction (SPE) with ENV+ cartridges (500 mg/6 mL from International Sorbent Technology Ltd., Hengoed, United Kingdom). The cartridges were cleaned prior to use using a vacuum filtration manifold as follows: 12 mL of dichloromethane (DCM) were passed through the cartridge followed by 12 mL of acetone and 12 mL of carbon-free water. The filtered water samples were passed through the cartridges, which were next dried under N_2 flow and stored at −20 °C until elution. The analytes were eluted with 6 mL of DCM into a 15-mL graduated tube. The DCM extracts were exchanged to hexane by blow down with N_2 and derivatized with PFBC as described by Datta et al. (*19*). Prior to analysis by GC/MS, 10 μL internal standard (polychlorinated biphenyl (PCB) 204) was added to each sample.

Sediment Preparation Procedures. The sediment extraction procedure for this study was a modification of an accelerated solvent extraction (ASE) and SPE cleanup procedure developed by Shang et al. (*2*, *28*). It was fully described in ref *27*. The method involved extraction of 1 g of uniformly ground and dried sediment using the ASE apparatus (ASE 200 from Dionex Corp., Sunnyvale, CA) with 1+1 acetone/hexane. The extracts were cleaned up using a stacked, multilayer modified SPE cartridge. The stack employed a 10-mL, 500-mg, BondElut aminopropyl silica cartridge (Varian Associates Inc., Harbor City, CA), 1 g of HCl-activated copper, and 3 g of previously baked Na_2SO_4. The stacked cartridge was precleaned using DCM and hexane; the sample was loaded dropwise, the unit was rinsed with hexane, and finally the APEs were eluted in acetone. Each eluate was reduced in volume by evaporation, and the solvent was exchanged to methanol/water (50/50) with a final volume around 0.5 mL. Prior to LC/MS-MS (Liquid Chromatography/Mass Spectrometry-Mass Spectrometry) injection, each extract was filtered through a hydrophilic polyvinylidene membrane. A ^{13}C-labeled internal standard mix ($^{13}C_6$-NP, $^{13}C_6$-NP(1.6) EO) was added just prior to injection.

Carp Preparation Procedures. *Sample Homogenization, Extraction, and Cleanup*. Each whole fish was ground and analyzed separately. Details of this procedure were reported in Datta et al. (*19*). The final product was a fairly uniform even-flowing smooth paste that was stored frozen at −20 °C in 300-mL capacity certified chemically cleaned glass jars fitted with Teflon-lined caps. The extraction procedures were modified from those of Datta et al. (*19*). Briefly, 7 g of the fish, after being thawed, were mixed with 28 g of baked sodium sulfate using a marble mortar and pestle. This entire mix was transferred to a 33 mL stainless steel ASE cell fitted with

cellulose filters (Soxhlet extracted with DCM to remove NP) at the bottom of the cell. After the ASE extraction with DCM, the extracts were evaporated and the solvent exchanged to hexane. These final extracts were adjusted to precisely 7 mL in hexane. A lipid determination was carried out on 0.5 mL of this extract. Aminopropyl cartridges (APS − 500 mg, 3 mL LC-NH_2 Supelco, Bellefonte, PA) were used to remove lipids from the fish extract (*19*). Fish extracts were divided into three equal portions, and each aliquot was loaded onto a separate APS cartridge which was conditioned as follows: rinsed 3 times with 3 mL of acetone, dried, and then further rinsed with 3 mL of DCM and finally, slowly rinsed with 3 × 3 mL hexane. After loading the fish extract, an additional 1-mL rinseate was added. Then 3 mL of hexane was used to wash the cartridge. Finally, the cartridge was eluted with 7 mL (90/10) of hexane/2-propanol to release the APEs. The eluants from the three cartridges were then pooled and N_2-evaporated down to exactly 4 mL with hexane as the final solvent. Based on several replicate determinations this cartridge cleanup method was found to remove 79% of contaminating interferences from the carp extracts.

Instrumental Methods. *HPLC/Fluorescence Analysis (Used for Fish Samples Only).* The method involved separation of the 25 μL injection volume on an aminopropylsilica normal phase column, 4.6 mm i.d. × 100 mm, 5 μm particle size (Hypersil APS from Agilent, Wilmington, DE) together with a similar-phase aminopropyl guard column 4 mm × 3.0 mm i.d. (Phenomenex, Torrance, CA), both kept at 23 °C. The separation was accomplished using a gradient mobile phase of hexane/2-propanol, starting at 98% hexane. The sample vials were capped with Teflon lids since standard plastic caps were introducing NP contamination. Fluorescence detection was achieved by 230 nm excitation wavelength and monitoring a 300 nm wavelength emission, with default slit widths of 18 nm used for each (standard settings for Model 474 Waters Scanning Fluorescence Detector). The internal standard, 4-propylphenol, at 70 ng/mL in the injected extract, was used to determine the relative retention times to identify the compounds. This was especially helpful for identification of compounds in the fish when matrix interferences were prevalent. Quantification was carried out by external standard methods. The elution order for the homologue mix was as follows: NP1EO, NP2EO, NP, NP3EO, NP4EO, and then NP5EO. The minimum detection limits (mdl) for the NPE's (ng/g fresh wt) using this method were as follows: NP, 5; NP1EO, 8; NP2EO, 20; NP3EO, 21; NP4EO, 32; and NP5EO, 44 (originally determined by Datta et al. (*19*)). It was not possible to chromatographically separate the nonyl homologues from the octylphenol group of compounds; this precluded us from quantitating the octylphenols by LC-Fluorescence methods.

GC/MS Conditions (Used for Water Samples Only). The GC/MS method employed here was adapted from Datta et al. (*19*). Briefly the conditions were as follows: The halogenated derivatives of the phenols were produced by reaction with PFBC, which were selectively determined using negative chemical ionization (NCI) detection. The standard for the alkylphenol analyses was a combined mixture of NPEs and OPEs (NP/OP, NP/OP 1 to 3 EO), which were derivatized with each batch of samples. The standard had to be prepared with progressively higher concentrations of the homologues as their ethoxy substitution increased. This was necessary to compensate for the decreasing sensitivity of the instrument as the APE's molecular weights increased. The derivatized APEs were analyzed by NCI/GC/MS using a Hewlett-Packard 5890A gas chromatograph and Hewlett-Packard 5989A mass spectrometer. A J&W Scientific DB-17MS column (30 m × 0.25 mm i.d., film thickness of 0.25 μm) was employed. Specific instrumental operating conditions are described in Datta et al. (*19*). The compounds of interest were identified using single ion monitoring (SIM) dwelling on the following ions: OP m/z = 400, NP m/z = 414, OP1EO m/z = 444, NP1EO m/z = 458, OP2EO m/z = 488, NP2EO m/z = 502, OP3EO m/z = 532 and NP3EO m/z = 546, and m/z = 430 for the internal standard PCB 204. Quantification was accomplished using the internal standard mode, relating everything to PCB 204. The minimum quantification limits for this method for OPEs and NPEs (ng/L) in water were as follows: OP, 0.1; OP1EO, 0.95; OP2EO, 3.8; OP3EO, 37 and NP, 1; NP1EO, 9; NP2EO, 40; NP3EO, 400.

HPLC/MS/MS Analysis (Used for Quantitation of the Sediment and for Confirmation of NPEs and Quantitation of OPEs in Selected Fish Sample Extracts). Chromatographic separation was performed on a Waters 2690 XE separations module (Waters Corp., Milford, MA). Ten microliters of sample were injected into an MSpak GF-310 4D column, 4.6 mm i.d. × 150 mm (Shodex, Shoko Co., Tokyo, Japan) at 60 °C. A size-exclusion guard column 4 mm × 3.0 mm i.d. (GFC-2000 from Phenomenex) was used to protect the column from contamination by dirty extracts. A mobile-phase gradient was necessary to separate the compounds using solvent A (1:1, 10 mM ammonium acetate water:methanol (MeOH)) and solvent B (MeOH). The gradient program went from 100% A to 90% B in 20 min, where it was held for 8 min and then programmed upward to 100% B over the next 2 min. The column was flushed with 100% methanol for 5 additional min and then returned to the starting conditions in 5 min and stabilized for another 20 min before the next run. The flow rate was set at 0.2 mL/min, and all of the column flow was allowed to enter the MS.

Electrospray mass spectrometry analysis was performed on a benchtop triple quadrupole mass spectrometer (Quattro LC from Micromass Ltd., Manchester, United Kingdom). Acquisition was done in the multiple-reaction monitoring mode (MRM) in electrospray positive (ES+) for the first 25 min of the run and then switched to electrospray negative (ES−) for 10 min. For a more detailed description of the operating parameters, refer to ref *27*. Briefly the positive ionization parent ions were the ammonium adducts of the analytes leading to specific daughter ions; the negative ionization parent ions were $[M-H]^-$ ions leading to specific daughter ions. Quantitation was performed by internal standard method using C_{13}-labeled homologues of the corresponding NP analytes, e.g., $^{13}C_6$-NP and $^{13}C_6$-NP1EO to $^{13}C_6$-NP4EO for both the NPEs and OPEs group of compounds. The minimum detection limits for the OPEs and NPEs in sediment (ng/g dry wt) were reported by Loyo et al. (*27*) as follows: OP, 1.4; OP1EO, 0.8; OP2EO, 0.08; OP3EO, 0.03; OP4EO, 0.02; OP5EO, 0.02 and NP, 0.9; NP1EO, 0.8; NP2EO, 0.1; NP3EO, 0.03; NP4EO, 0.03; NP5EO, 0.03 and with fish for the OPEs they were reported by Schmitz-Afonso et al. (*29*) as minimum quantification limits (ng/g fresh wt) as follows: OP, 20; OP1EO, 20; OP2EO, 6; OP3EO, 6; OP4EO, 6; OP5EO, 6.

Quality Control Issues. *Fish Quality Control Performance.* Because of the high levels of interferences often remaining in the fish extracts it was found to be very important to have the quantitation standards prepared in fish extracts of clean carp samples. This was accomplished by making up a standard curve for each sample batch using clean matrix materials from the blank carp samples (e.g., carp materials from site #1 were used) that were run with each batch. Therefore any interactions between coextractive fish lipids in samples and individual analytes should be similar to those same effects arising in the calibration curves. Procedural blanks showed no interference except NP at a level of 7 ng/g, which was below the method quantification limit (MQL).

Recovery experiments were carried out by spiking clean carp (site #1) samples. Spike recoveries were consistently acceptable for all of the ethoxylates (ranging from an average

TABLE 2. Total Alkylphenol and Alkylphenol Ethoxylates (t-NPE and t-OPE, 0−5 Ethoxy-Substituted)[a] Detected in Each Sample Type and at Different Locations on the Cuyahoga River

	water concentrations (n = 1 per site) (μg/L)		sediment concentrations (n = 1 per site) (μg/kg dry wt)		average fish concentrations (n = number of fish per site) (μg/kg wet wt)	
river site	t-NPE	t-OPE	t-NPE	t-OPE	t-NPE	t-OPE
site 1	0.13	0.0053	250	74	32 (11)	none analyzed
site 2	0.24	0.0085	830	20	140 (11)	none analyzed
site 3	1.8	0.048	230	49	150 (12)	106 (1)
site 4	0.19	0.0097	640	56	160 (12)	54 (1)
sites 5/6[b]	5.0, 5.1	0.16, 0.19	440, 480	67, 61	920 (11)	18 (2)
site 7	1.0	0.016	1020	62	550 (12)	25 (2)
site 8	0.34	0.012	1010	53	490 (12)	36 (2)

[a] See text for compositional makeup for total NP 0 to 5 EO and OP 0 to 5 EO in each matrix. [b] This site was the field duplicate site for water and sediment.

of 78% to 93% (n = 22) and precision <22% RSD (relative standard deviation). The ethoxylate method was also found to be reproducible based on 15 duplicate pairs e.g., RSD = 18%; however, for NP the RSD was 63%. Furthermore, recovery for nonylphenol was low averaging only 44%; with an RSD of 60% RSD. Had the low NP recoveries been more reproducible it might have been possible to apply a correction factor; however, this could not be justified. Subsequent research has now been carried out to investigate the NP recovery problem (*29*). The findings suggested that there is enough differences in the tissues of the two fish, e.g. carp and lake trout (the fish that the original method was validated with (*19*)), that selecting a different solvent for the extraction of the carp (acetonitrile vs methylene chloride) would correct the problem.

LC/MS/MS spectrometry was employed on 10% of the fish sample extracts to confirm the results produced by the LC-fluorescence method. These analyses also provided data on octylphenols in these fish. The analytical method was similar to that used for the sediment except that an additional cleanup was employed involving C-18 solid-phase chromatography (*29*). The average LC/MS-MS quantification results for each homologue group were all within 83% of the original fluorescence values in addition to detecting traces of NP3EO (9−24 ng/g wet wt).

Water and Sediment Quality Control Results. For the water samples, the recoveries were all acceptable (e.g., greater than 70%). Reproducibility of the method was also acceptable; the relative percent differences of the duplicate pairs were all less than 9% for NPEs and they averaged 11% for the OPEs. For sediment samples, the average recoveries for NP and the NP-ethoxylates varied from 70 to 115%, and for the OPEs (including octylphenol), they varied from 65 to 106%. With the sediment samples, each was analyzed in duplicate, and the average for the two determinations used in order to compensate for the high natural variability between duplicate analyses of sediment samples. The average relative percent difference for these pairs was 37%.

Statistical Assessment of the Fish Residue Data. The data were screened statistically in order to attempt to better explain the variability of the NPE concentrations observed in the fish and attempt to relate this with sampling locations; other relationships could have been tested, especially sex differences; however, these findings will be dealt with in a subsequent paper. Standard statistical processing was performed using a SAS systems approach, e.g. SAS Software and SAS Institute Inc. methods (*30*).

Results and Discussion

There was clearly an increase in total alkylphenols (especially the nonyl group of alkylphenols) in water, fish, and sediment, in going from the nonurbanized, rural portion of the river, MP 83.93, to the urbanized and industrialized segments (Akron, MP 42.73, and Cleveland, MP 10.35) (Table 2 and Figures 2−4). The concentration of total nonylphenols in water and fish reached its maximum at MP 35.31, which is 2.1 miles downstream of the Akron WWTP outfall. Total octylphenol concentration in water was also highest at the Akron WWTP outfall site, Table 2. The discussions below provide the exact compositional makeup for these totals of NP 0 to 5 EO and OP 0 to 5 EO in each of these sample types. The percentage of total APE that belonged to the octylphenol family averaged 3.3% in water samples and 10.6% in sediment and ranged from 1.6 to 42% in fish. The sediment concentrations of the lower ethoxymers of NP (NP 0 to 5 EO) were highest near the city of Cleveland where the Cuyahoga River discharges to Lake Erie.

It appears that the higher levels in fish and to a lesser extent in the water are linked to WWTP discharge and increases in urbanization. The highest levels were 2.1 miles downstream of the Akron WWTP, and there was also an indication of an increase in water concentration at site 3 which is where the Akron CSO contributions are heaviest, MP 45.1 to 41.3 (*24*). There however does not appear to be any increases in APEs in water or fish coming from added pollution that is believed to enter from the Little Cuyahoga, cf. site 3 versus site 4. With sediment however a slight increase was observed. These differences could indicate possible pollution from hazardous waste sites along the Little Cuyahoga and a heavy input of past CSO discharges from this River. CSO contributions of APEs to the water concentrations during this period were probably not that important since the most recent rain event occurred 5 days prior to the sampling e.g., 22.6 mm on July 3rd and the next closest rain occurred 5 days earlier, 4.3 mm on the 29th of June.

Fish. The average total concentrations of nonylphenol and nonylphenol ethoxylates (NPEs) found in the fish varied from 32 μg/kg at the most upstream "clean" site to a high total NPE value of 920 μg/kg near the Akron WWTP plant outfall, Table 2. In nearly all cases, the total NPE concentrations were predominantly composed of the NP1EO homologue (average was >59% of the total NPE result), and the NP2EO form averaged 22% of the total. Only random fish had traces of the ethoxymers above NP2EO, and these were detectable only with the MS-MS method. The average NP concentration in fish samples for the most upstream, "clean" site was 7 μg/ kg wet weight, which was the lowest nonylphenol concentration in fish from all sites sampled. The data reported by Keith et al. (*20*) for the Kalamazoo River, MI, might be considered comparable. They only detected nonylphenol in 59% of the fish in their survey of 183 mixed species of fish (not including carp). They reported an average concentration of 4 μg/kg NP across all sites, including the nondetects, and did not measure any NP1EO or NP2EO above

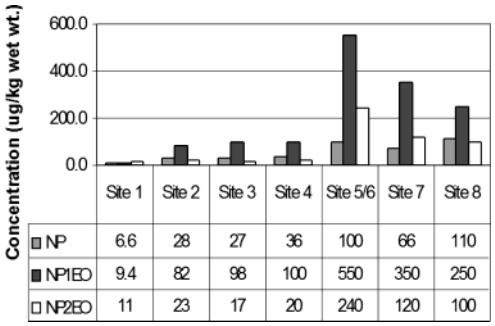


FIGURE 2. Distribution patterns for average nonylphenol (NP) and nonylphenol ethoxylate (NP1 and NP2EO) in carp samples collected from MP 83.93 (site 1) to MP 10.35 (site 8) on the Cuyahoga River, Ohio. Calculated as ln-normalized mean values.

their detection limits. Bennie et al. (*12*) reported values for nonylphenol in carp from Hamilton Harbor and Lake Ontario that were somewhat higher than these, ranging from <20 (detection limit) to 43 μg/kg. The fish from our "clean" site also had low levels of NP1EO; however, neither Bennie nor Keith found NP1EO in their fish, which may reflect their higher detection limits for this ethoxymer. For this study, the highest average values for individual NP-homologues in the fish were found at site 5/6 and site 8, downstream of the WWTPs. The average NP concentration was highest in fish collected at site 8, 110 μg/kg wet weight (Figure 2), whereas concentrations of NP1EO and NP2EO were highest in fish from site 5/6, 550 and 240 μg/kg wet wt, respectively.

The higher NP levels in fish samples from the Cuyahoga River (66−110 ug/kg wet wt) might be considered moderate when compared to maximum NP fish residue values that have been reported in the literature. For example in United States waters maximum values range from 184 (*21*) to 223−1842 (*19*) ug/kg wet wt and in Europe they range from 243 to 800 ug/kg wet wt (*17*). The ethoxylates, NP1EO and NP2EO, that were measured here are, however, among some of the highest reported for fish, especially in United States waters. Datta et al. (*19*) reported values that were higher in a single carp from the Detroit River at 2075, 567, and 402 μg/kg wet wt, respectively for NP1EO, NP2EO, and NP3EO, while the other two carp samples in their study had values similar to those found here. Snyder et al. (*21*) reported concentrations in carp from Lake Meade, NV that had an average of 242 μg/kg wet wt for NP1EO, but they found no NP2EO in these fish. One of the highest reported concentrations of APEs in fish is from Blackburn et al. (*17*) for fish from the River Aire in the United Kingdom. These results were an average of muscle tissue from three Gudgeon and three Roach collected in 1995 that ranged from 600 to 800 μg/kg wet weight for nonylphenol and 1400 to 4200 μg/kg wet weight for the sum of NP1EO and NP2EO.

For the octylphenols, only eight fish were analyzed; these data are shown as total octylphenols (the sum of ethoxymers 1 and 2) (ranging from 18 to 106 ug/kg wet weight) for sites 3, 4, 5/6, 7, and 8, Table 2. Octylphenol itself was not detectable in any of the fish above the MQL of 20 ng/g (*29*). Only OP1EO and OP2EO were detected, and OP1EO constituted > 74% of the total OPE in fish tissue samples from all sites except site 5/6 where OP2EO was higher. The average percentage of total octylphenol compared to the total nonylphenol in these fish was 10%, which is similar to the relative usage of these two nonionic surfactants in commerce (*31*). There are fewer OPE data in the literature to compare our values against. Ferrara et al. (*32*) measured concentrations of octylphenol up to 18.6 μg/kg wet wt, and Bennet and Metcalfe (*33*) found 25 μg/kg wet wt (converted from their lipid normalized results) in caged mussels near the Detroit Sewage Treatment Plant outfall. Ferrara and associates (*32*) were the only researchers to report OP1EO; however, their samples had only 0.08−0.43 μg/kg of this homologue.

TABLE 3. Statistical Analysis of the ln-Normalized Mean Total Values of NPEs in Fish without Distinguishing Sex

site	mean total NPE (μg/kg)	site	mean total NPE (μg/kg)
1	31.5D[a]	5/6	919B
2	142C	7	546AB
3	146C	8	487A
4	165C		

[a] Site means with different A−D letters are different at 0.05 significance level.

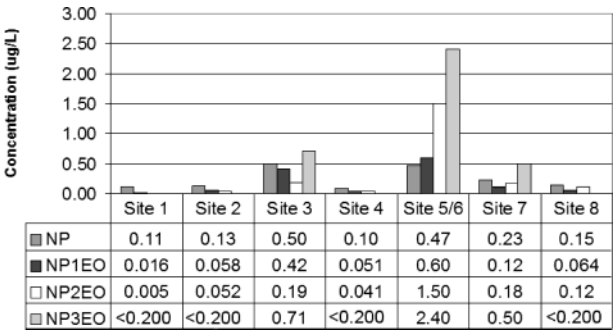


FIGURE 3. Distribution patterns for nonylphenol (NP) and nonylphenol ethoxylate (NP1 to 3EO) in water samples collected from MP 83.93 (site 1) to MP 10.35 (site 8) on the Cuyahoga River, Ohio (in those cases were no NP3EO was detected, the estimated amount was listed as less than half the limit of quantitation).

Since a large number of fish were measured at each location, it was possible to carry out statistical correlations for the NPE group with other data on the fish. An analysis of variance was performed initially on all of the data where it was determined that most of the variability in total NPE concentration could be attributed to station differences. Total nonylphenol (NP + detectable NP-ethoxylates) was evaluated with censored values (values below the method detection limit) replaced by $^1/_2$ the detection limit values. Total NPE was analyzed as a two-factor general linear model using PROC MIXED (SAS Institute Inc. (*30*)) with site as the factor. To correct for non-normality the values were ln(x) transformed. For each log-transformed variable, the residuals from the model were tested for normality with four goodness of fit tests (*32*), and the hypotheses of normality were not rejected. The mean comparisons were done with Sidak adjusted p-values so that experiment-wise error was 0.05. The average concentration in fish at site 1 is significantly lower (0.05 significance level) for total NPE than all the other sites (Table 3). Sites 2, 3, and 4 are next highest as a group and all were significantly lower than sites 5/6, 7, and 8. In Table 3 it is demonstrated statistically that sites 5/6 and 8 are similar and significantly higher in total NPE than all of the other sites. Potential sources of NPE upstream from these sites include WWTP from Akron, site 5/6, and Cleveland Southerly, site 8, as well as urban and industrial areas of Cleveland, site 7.

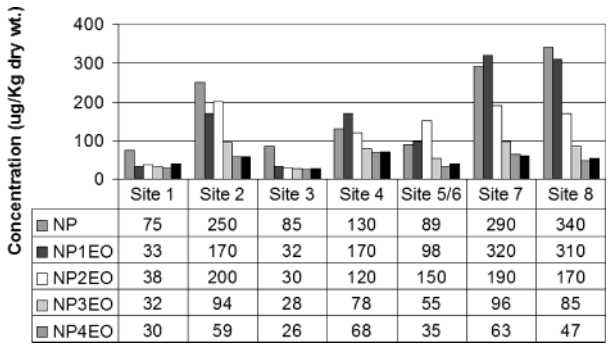
Water. As noted above, the pattern for distribution of total NPEs over the studied portion of the river agreed with the fish results in that the highest total NPE concentration occurred at site 5/6, average of 5.0 μg/L (Table 2). It is interesting to note that at all of these locations the predominant NP-homologue is the 3-ethoxymer (Figure 3); NP2EO was next in abundance. The fact that this 3-ethoxymer was not a major contributor to the total nonylphenol concentration of either the fish or sediment collected at these stations, however, suggests that discharge of the 3-ethoxymer

may not have been a continuous occurrence at these sites. Another possibility would be that it is not readily accumulated by fish or degrades more rapidly than the other ethoxymers and therefore disappears before accumulating in sediment or fish.

The concentration of total OPEs in water varied in a pattern very similar to the distribution of NPE over the river, even down to the fact that there was a slight increase in concentration at site 3 (Table 2). However, rather than the 3 ethoxymer dominating at any of the sites, it was OP2EO that dominated and OP3EO was next in abundance. The higher relative abundance of the 1−3 ethoxymer pattern for this octyl group at site 3 matches the same pattern observed for the nonyl group at this site and provides further support for a local point source discharge of APEs between sites 2 and 3. Potential sources in this reach of the river would be the extensive urbanization that surrounds the Cuyahoga just before site 3 and extends about one-third of the way to site 2 (see population density map, Figure 1, in ref *34*). As mentioned above, the source then could be from very local combined storm overflows (CSO) discharges of domestic and commercial waste in these urban areas since few hazardous waste sites exist in this reach of the river (*24*). It should be cautioned that our water values were but a snapshot in space (only one sample location was occupied over the width of the river) and time and should not be overinterpreted.

In comparing the total NPE values observed here to those reported in the literature, the highest values, 5.0 μg/L NPE and 0.23 μg/L OPE, are within the range of values that Barber et al. (*7*) determined for Midwest river samples collected near sewage treatment plant discharge sites. Barber's samples were generally dominated by NP1- and 2-ethoxymers with a few of them containing detectable quantities of the 3- and 4-ethoxymers. All the other Cuyahoga river locations had values that were lower in total NPE and OPE concentration. Two of these, site 3 (1.8 μg/L NPE and 0.048 μg/L OPE) and site 7 (1.0 μg/L NPE and 0.016 μg/L OPE) had intermediate water concentrations that approached values reported by Snyder et al. (*35*) (non WWTP data) and Kolpin et al. (*8*). There was comparability with our data in the methods used by Kolpin et al. who also used GC/MS; however, Snyder et al. employed LC/fluorescence methods which might have been more suitable for analyzing the higher ethoxymers, which did appear to result in total NPE values that were higher than ours. The detection limits (MDL) by Snyder and associates for the NP and NPEs in water were comparable to ours.

Sediment. The distribution of NPEs in sediment over the sampled regions of the Cuyahoga River is different than for the fish and water. For those homologues that were identified in the fish and water (ethoxymers 0−3), maximum values occurred at sites 7 and 8. At most sites it was the NP and NP1EO homologues that were generally present in greatest amounts (Figure 4). The sediment samples were also scanned (semiquantitatively) for higher ethoxymers (NP 6 to 17 EO). The LC/MS-MS identifications for ethoxymers 6−17 were compared to the homologue patterns in Igepal 720 (a mixture rated as a 12-ethoxylate containing polymer). Every site had a pattern of higher ethoxymer peaks very similar to the Igepal 720 standard. While the results must be considered only semiquantitative (full tests for spike and recovery of these higher ethoxymers were not carried out and the purity of the Igepal 720 was not checked), the significance of these findings is very important. For example, at all of the sites the higher ethoxymer contributions to total APE levels was greater than 28% and at site 4 and site 1 the percent contributions were 59 and 81%, respectively. As mentioned earlier, the presence of these higher ethoxymers of the APEs, e.g., greater than 3−5 ethoxy-substitution, suggests that unprocessed wastes are entering the river, and this fact needs further research.



	Site 1	Site 2	Site 3	Site 4	Site 5/6	Site 7	Site 8
NP	75	250	85	130	89	290	340
NP1EO	33	170	32	170	98	320	310
NP2EO	38	200	30	120	150	190	170
NP3EO	32	94	28	78	55	96	85
NP4EO	30	59	26	68	35	63	47

FIGURE 4. Distribution patterns for nonylphenol (NP) and nonylphenol ethoxylate (NP1 to 5EO) in sediment samples collected from MP 83.93 (site 1) to MP 10.35 (site 8) on the Cuyahoga River, Ohio.

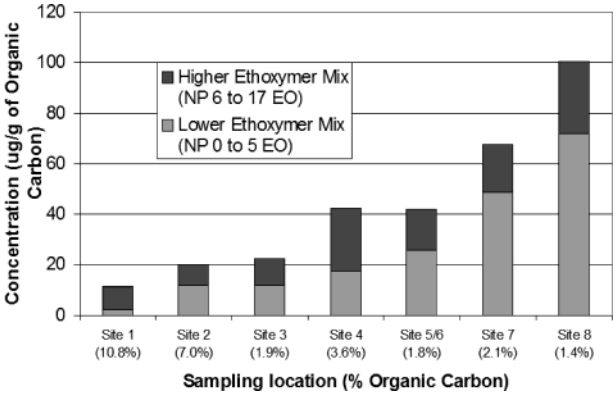


FIGURE 5. Amount of total NP 0 to 17 EO in sediment, normalized to organic carbon content at each site along the Cuyahoga River, Ohio.

Furthermore, finding these higher ethoxymers of NPEs in the sediments of this river reinforces the importance of including these in analytical schemes, especially those employed on sediments.

The organic carbon normalized concentrations of total NPEs (estimated higher ethoxymer plus lower ethoxymers) were plotted versus locations on the river, Figure 5. Along with describing the sediment distribution of NPEs this clearly demonstrates the importance of organic carbon to contaminant distribution in these samples. Normalizing these results to organic carbon (shown as percent organic carbon, Figure 5—data provided by Ohio EPA (*24*)) contributed greatly to reducing the variability in these data, e.g., compare Figures 4 and 5. Ferguson and associates (*15*) found a similar relationship for alkylphenol concentration in sediment of Jamaica Bay, NY. They suggested that this occurs because the APEs are primarily associated with organic-rich, fine-grained sediments. It is interesting to note that the trend for increasing total NPE sediment concentration per unit of organic carbon that is shown in Figure 5 matches a similar increase in urbanization/industrialization that takes place along the river in moving from site 1 to site 8.

The maximum concentration of total lower ethoxymer-containing (NP 1 to 5 EO) sediments was 1020 μg/kg dry wt at site 7, and for total OPEs the maximum was 74 μg/kg dry wt at site 1, Table 2. Separating out these values into their predominant homologues (NP, NP1 to 2EO), the maximum levels were as follows: NP, 340 μg/kg at site 8, NP1EO, 320 μg/kg at site 7, and NP2EO, 190 μg/kg at site 7. If these results are compared to published data for sediment throughout the United States and Canada, this river appears to have low to moderate levels of NPE and OPE. Much higher levels have been reported in upper Midwestern Rivers. For example,

in the Detroit and Rouge River Kannan et al. (*10*) measured 10−60 000 μg/kg dry wt for just NP and Bennet and Metcalfe (*14*) reported 200−37 800 μg/kg dry wt for NP to NP2EO at other sites in the Detroit River. For rivers in the Chicago area, Zintek et al. (*11*) found the North Branch of the Chicago River to have NP concentrations ranging from 2500 to 4800 μg/kg, NP1EO from nd to 49 000 μg/kg, and NP2EO concentration from nd to 16 000 μg/kg.

Even though the concentrations measured in sediment of the Cuyahoga River may be considered moderate they could be acting as a reservoir that continually exchanges with bottom feeding fish like the carp in this study. This was a concept suggested by Snyder et al. (*21*) as the means by which high levels of NP and NPEs were accumulating in carp in Lake Mead.

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Literature Cited

(1) Renner, R. *Environ. Sci. Technol.* **1997**, *31*, 316A−320A.
(2) Takada, H.; Eganhouse, R. P. Molecular Markers of Anthropogenic Waste. In *Encyclopedia of Environmental Analysis and Remediation*; Meyers, R. A., Ed.; John Wiley & Sons: New York, 1998; pp 2883−2940.
(3) Shang, D. Y.; Ikonomou, M. G.; MacDonald, R. W. *J. Chromatogr. A* **1999**, *849*, 467−482.
(4) Jobling, S.; Sumpter, J. P. *Aquat. Toxicol.* **1993**, *27*, 361−372.
(5) Environment Canada. Priority Substances List Assessment Report, Nonylphenol and Its Ethoxylates. Environmental Protection Publications: Environment Canada, Ottawa, Ontario, 2001.
(6) Hale, R. C.; Smith, C. L.; de Fur, P. O.; Harvey, E.; Bush, E. O.; La Guardia, M. J.; Vadas, G. G. *Environ. Toxicol. Chem*. **2000**, *19*, 946−952.
(7) Barber, L. B.; Brown, G. K.; Zaugg, S. D. Potential Endocrine Disrupting Organic Chemicals in Treated Municipal Wastewater and River Water. In *Analysis of Environmental Endocride Disruptors; In Analytical Methods for Endocrine Disrupters*; Keith, L. H., Jones-Lepp, T. L., Needham, L. L., Eds.; Washington, DC, 2000; pp 97−123.
(8) Kolpin, D. W.; Furlong, E. T.; Meyer, M. T.; Zaugg, S. D.; Barber, L. B.; Buxton, H. T. *Environ. Sci. Technol.* **2002**, *36*, 1202−1211.
(9) Harries, J. E.; Sheahan, D. A.; Jobling, S.; Matthiessen, P.; Sumpter, J. P. *Environ. Toxicol. Chem*. **1997**, *15*, 1993−2002.
(10) Kannan, K.; Kober, J. L.; Kang, Y.-S.; Masunaga, S.; Nakanishi, J.; Ostaszewski, A.; Giesy, J. P. *Environ. Toxicol. Chem*. **2001**, *20*, 1878−1889.
(11) Zinteck, L. B.; Parachuri, B.; Wesolowski, D. J. Society of Environmental Toxicology and Chemistry (SETAC 22nd Annual Meeting), Abstract Book, 131. 2001.
(12) Bennie, D. T.; Sullivan, C. A.; Lee, H.-B. M. R. J. *Water Qual. Res. J. Can.* **1998**, *33*, 231−252.
(13) Naylor, C. G.; Mieure, J. P.; Adams, W. J.; Weeks, J. A.; Castaldi, F. J.; Ogle, L. D.; Romano, R. R. *JAOCS* **1992**, *69*, 695−703.
(14) Bennett, E. R.; Metcalfe, C. D. *Environ. Toxicol. Chem*. **1998**, *17*, 1230−1235.
(15) Ferguson, P. L.; Iden, C. R.; Brownawell, B. J. *J. Chromatogr. A* **2001**, *938*, 79−91.
(16) Ahel, M.; Schaffner, C.; Giger, W. *Water Res.* **1996**, *30*, 37−46.
(17) Blackburn, M. A.; Kirby, L. J.; Waldock, M. J. *Mar. Pollut. J.* **1999**, *38*, 109−118.
(18) Lye, C. M.; Frid, C. L. J.; Gill, M. E.; Cooper, D. W.; Jones, D. M. *Environ. Sci. Technol.* **1999**, *33*, 1009−1014.
(19) Datta, S.; Loyo-Rosales, J. E.; Rice, C. P. *J. Agric. Food Chem*. **2002**, *50*, 1350−1354.
(20) Keith, T. L.; Snyder, S. A.; Naylor, C. G.; Staples, C. A.; Summer, C.; Kannan, K.; Giesy, J. P. *Environ. Sci. Technol.* **2001**, *35*, 10−13.
(21) Snyder, S. A.; Keith, T. L.; Naylor, C. G.; Staples, C. A.; Giesy, J. P. *Environ. Toxicol. Chem*. **2001**, *20*, 1870−1873.
(22) Kannan, K.; Keith, T. L.; Naylor, C. G.; Staples, C. A.; Snyder, S. A.; Giesy, J. P *Arch. Environ. Contam. Toxicol.* **2003**, *44*, 77−82.
(23) Ohio EPA. Biological and Water Quality Study of the Little Cuyahoga River and Tributaries, 1996; Summit & Portage Counties. 1998, Technical Support Document MAS/1997-12-9. Division of Surface Water, Columbus Ohio. (http://www.epa.state.oh.us.)
(24) Ohio EPA. Preliminary Assessment/Site Inspection (PA/IS) Sampling Investigation Final Report for Cuyahoga River, A Study of Biomarkers, Fish, Sediment and Surface Water of the Cuyahoga River. USEPA ID: OHS FND 508 019, 2002. 20 pp.
(25) Ohio EPA. Fish tissue guidance manual. Division of Surface Water, Monitoring and Assessment Section. Ohio EPA Fish Tissue Guidance Manual. 94. Columbus, Ohio, Ohio Environmental Protection Agency. Ohio EPA, Technical Bull. MAS/1994-11-1.
(26) Ferguson, P. L.; Iden, C. R.; Brownawell. B. J. *Anal. Chem*. **1999**, *72*, 4322−4330.
(27) Loyo-Rosales, J. E.; Schmitz-Afonso, I.; Rice, C. P.; Torrents, A. *Anal. Chem*. In press.
(28) Shang, D. Y.; MacDonald, R. W.; Ikonomou, M. G. *Environ. Sci. Technol.* **1999**, *33*, 1366−1372.
(29) Schmitz-Afonso, I.; Loyo-Rosales, J. E.; Avilés, M. P.; Rattner, B. A.; Rice, C. P. *J. Chromatogr. A* In press.
(30) SAS Institute Inc. *SAS/STAT User's Guide*; SAS Institute Inc.: Cary, NC, 1999.
(31) Pedersen, R.; Hill, E. M. *Environ. Sci. Technol.* **2002**, *36*, 3275−3283.
(32) Ferrara, F.; Fabietti, F.; Delise, M.; Piccioli Bocca, F. E. *Environ. Sci. Technol.* **2001**, *35*, 3109−3112.
(33) Bennett, E. R.; Metcalfe, C. D. *Environ. Toxicol. Chem*. **2000**, *19*, 784−792.
(34) Ohio EPA. Total Maximum Daily Loads for the Middle Cuyahoga River. Ohio Environmental Protection Agency, Division of Surface Water, Columbus, Ohio. 2000. Appex. A to F, 212 pp.
(35) Snyder, S. A.; Keith, T. L.; Verbrugge, D. A.; Snyder, E. M.; Gross, T. S.; Kannan, K.; Giesy, J. P. *Environ. Sci. Technol.* **1999**, *33*, 2814−2820.

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